mones. It is possible that the lack of response to the hormones is a result of the destruction of a portion of the central nervous system which is indispensable to the integration of a complex behavior pattern. If further control experiments prove this to be the case, then the possibility must be considered that the integrating mechanism involved is located in the midventral portion of the anterior hypothalamus instead of the region of the mammillary bodies or the mesencephalic tegmentum.

11356

Selective Localization of Evans Blue (T1824) in Subplacental Portions of Entoderm in the Rat.*

ALEXANDER BRUNSCHWIG, ROBERT L. SCHMITZ AND SHERMAN JENNINGS.

From the Department of Surgery, The University of Chicago.

The dye, Evans blue (T1824), a non-toxic, azo-compound, isomer of trypan-blue, has been observed following intravenous injection to localize selectively in and about malignant neoplasms in animals[1] and in man[2] but does not localize selectively in and about benign tumors in man or animals.[2] In and about the malignant neoplasms it accumulates in the macrophages and fibroblasts of the stroma. It does not penetrate into the neoplastic cells themselves, whether they be carcinoma or sarcoma.

During the course of experiments to observe its localization in tumor-bearing rats a pregnant animal (about mid-term) was inadvertently employed. At necropsy 24 hours after intravenous injection of 4 mg of the dye dissolved in 1 cc distilled water, it was noted that in addition to the sarcoma there was marked selective concentration of the dye in that portion of the visceral entoderm subjacent to the disc-shaped placenta. The remainder of the visceral entoderm did not appear grossly to have localized the dye (Fig. 1). The uterine musculature appeared tinged lightly blue as did the placenta; the embryo, and the amniotic fluid contained no dye grossly visible.

To confirm these observations 7 pregnant female white rats were

* This work was carried out under a grant from the Cancer Research Institute of the Chicago Woman's Club, Chicago, Illinois.

1 Duran-Reynals, F., *Am. J. Cancer*, 1939, **35**, 98.

2 Brunschwig, A., and Clarke, T. H., *Am. J. Path.*, in press.

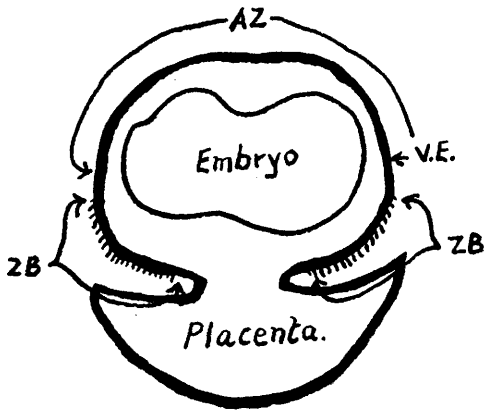

FIG. 1.

Diagram of cross-section of rat embryo in latter part of gestation. VE, visceral entoderm. ZB, subplacental portion of visceral entoderm with villous-like processes. These processes are not visible macroscopically. Selective localization of Evans blue was noted in this zone. AZ, anti-placental portion of visceral entoderm; cells are relatively lower in this region and no localization of the dye was noted here.

injected with similar quantities of dye, some at mid-term, and some just prior to the expected date of delivery. The animals were killed and at necropsy, 24 hours after injection, the concentration of the dye was observed in each instance as described above. Six pregnant females were injected with 4 mg of trypan blue. In 3 the localization occurred in the subplacental portion of entoderm as was found for Evans blue; in one the localization was not intense in this area, and in 2 it did not occur at all. Two pregnant females received intravenous injections of .5 cc India ink (diluted with water 1 to 3) and the latter localized definitely within the placenta as well as in the liver, spleen, bone marrow and some lymph nodes. The entire entoderm remained free from India ink.

Microscopic study of sections of the excised entoderms, portions of which had concentrated the Evans blue, and which were rapidly fixed, dehydrated and embedded to prevent loss of dye by diffusion, showed the dye within the columnar entoderm cells themselves as small aggregates of blue granules. The subplacental portions of the visceral entoderm are composed of tall columnar cells thrown into villus-like processes. The cells of the visceral entoderm became progressively flatter away from the placenta until over the area opposite the placenta where no dye was concentrated they are very low, flattened cells.

Discussion and Summary. The above described selective localization of Evans blue is of special interest since this localization in epithelial cells is in contrast to that observed when the dye concentrates selectively in and about carcinomas or sarcomas which localiza-

tion is in mesoblastic cells of the stroma—*i. e.,* fibroblasts and macrophages. Such selective concentration might denote a special physiologic property of a portion of the visceral entoderm in the type of placentation represented in the rat.

Goldmann[3] in his studies on intravital staining showed that trypan blue was concentrated in all of the entoderm in mice. However, storage of this dye was also observed by him in all of the reticuloendothelial system, of the mother, because of the relative enormous doses of the dye administered. Such very large doses precluded the demonstration of selective affinity for the dye on the part of certain tissues, as for example, the subplacental visceral entoderm. In his excellent monograph entitled "The Localization of Disease" Burrows[4] illustrates in colors the exposed viscera of a pregnant rat injected with isamine blue. The uterine horns have not been opened and the dye appears localized in and about the placenta. The illustration was published to indicate specific concentration of the dye in the placenta. From our own studies in which the gross appearance of the unopened pregnant uterus was similar to that depicted by Burrows, we would raise the question of whether the dye he used was also concentrated not in the placenta primarily, but rather in the subjacent visceral entoderm as occurred with Evans blue in the observations reported above.

11357 P

Use of Orally Administered Desiccated Thyroid in Production of Traumatic Shock.

R. J. SCHACHTER AND J. HUNTINGTON. (Introduced by N. Kleitman.)

From the Department of Physiology, University of Chicago.

In an attempt to study the effect of adrenal cortical extracts on traumatic shock, we observed that normal anesthetized dogs, following repeated trauma to the limbs, testicles and gut, did not go into shock at least within 8 hours. We discussed this problem with Dr. Ivy,[1] who had produced shock in dogs by trauma, and he suggested that a possible reason for his ability to induce shock in dogs might

3 Goldmann, E., *Beitr. zur Klin. Chirurg.*, 1909, **64**, 192.

4 Burrows, H., *Localization of Disease*, Wm. Wood & Co., London, 1932.

1 Ivy, A. C., *Am. J. Physiol.*, 1920, **51**, 197.